The Biology of Parasitic Spirochetes

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Preface

The purpose of this book and the symposium from which it arises is to present an overview and the current information available on the parasitic spirochetes. The etiological agents of the treponematoses, leptospiroses and the relapsing fevers are discussed by distinguished investigators who provide extensive and sometimes unique knowledge of these bacteria. In addition to providing a valuable resource of information, this volume should reveal the gaps in our knowledge and stimulate interest in some of the neglected areas of spirochetal biology.

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SOME PERSPECTIVES FOR THINKING ABOUT SPIROCHAETAL STRUCTURE

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The spirochaetes had the advantage over us for many years: They were so thin that components were hard to resolve; they had attractively sinuous shapes, and some of them produced remarkably protean diseases, and they were not easy to grow. It was no wonder that the serious student of their activities felt strongly that they were no ordinary microbe and considered them a breed apart. Perhaps the form of some of the larger saprophytic and free-living spirochaetes, the staining properties, the somewhat odd motility and the flexuous behaviour of the whole group suggested to a few that they might be related to the protozoa. However, intuition and the methods required for their study kept them in the bacteriological fold until comparative biochemistry and electron microscopy made the alliance definite.

There were few morphological characters, but the presence of non-pathogenic treponemes in the mouth and other places put the onus on microscopists of those early days to seek and be aware of small distinctions. Wavelengths of the primary coils were assiduously measured, terminal spires, axial filaments and patterns of movement were described. These things are hard enough to resolve with today's microscopes and the help of phase contrast or interference microscopy, which brings to mind a statement of exasperation at the observational capability of our forebears: "Maybe the wavelength of light was shorter then." In recent decades we have, of course, effectively shortened the wavelength and improved resolution by using electrons instead of photons for microscopy. Electron microscopy and the all-important methods of preparation of specimens now allow examination of not only whole organisms, globally and in section, but of component parts as they are revealed in the surfaces of cleavage planes or fractionated and separated by centrifugation, down to the shape and form of macromolecules as they lie embedded in negative stains. Perhaps experience with high-voltage microscopes

will allow new perceptions without breaking the integrity of these small cells. Biological materials seldom allow one to even approach theoretical resolution but the practical range of 1.0 - 2.5 nm gets us into the macromolecular range and provides us with enough mysteries.

Why is ultrastructure so important? It is not possible to make a realistic description of any microbe at any hierarchical level without including the structural attributes of the cell or cells that make it up. Sections show clearly that the spirochaetes are procaryotic and have the unique features of nuclear structure distinguishing the Procarotae from the rest of the living world. There is nothing inconsistent with this in any other aspects of cellular structure, e.g. disposition of membranes and wall layers, ribosomal dimensions, chemical characteristics of the mucopeptide, and the presence of flagella. These latter are usually called axial fibrils by those who study spirochaetes, but they are flagella, in comparative terms, and not modified to any great extent even if the utilization is strange. A major distinction of the group is that spirochaetes retain these flagella within the wall and dispose them along the axis of the helix formed by the protoplasmic cylinder. This is one stage removed from that in some vibrios and spirilla, where the "outer membrane" (LPS layer) of these Gram-negative cell walls is carried over the whole length of the flagella shaft forming a sheath. There is also another form of "sheath" on some flagella, including those of some spirochaetes, which is an assembly of macromolecules outside the helical packing of flagellin forming the shaft.

Modern cytology attempts to correlate structure, chemical nature and physiological function. No cellular activity has been more frustrating to this ambition than the function of flagella and understanding of the swimming motility of bacteria despite much solid work and some ingenious hypotheses. No less difficult, and probably related in kind, is the peculiarly flexuous and spinning motility of the spirochaetes. However, it would still be a reasonable article of faith to maintain that the axial fibrils of spirochaetes have some direct involvement in motility. Observations of fundamental importance contributing to the resolution of this mystery could well be made on organisms such as Leptospira with their remarkable "button-hook" ends as rotation markers.

It may be that the mucopeptide is tightly attached to the outer surface of the plasma membrane, an uncommon feature in other bacteria, but the general construction of the cell wall resembles that of the Gram-negative bacteria. It is believed there is a "space" between the plasma membrane and the outer

membrane, (i.e. between two major diffusion barriers) including the mucopeptide of Gram-negative organisms, which contains a variety of loosely held macromolecules including enzymes and is called the periplasmic space. Presumably there is an equivalent space of as much or more importance to the pathogenic spirochaetes.

The outer membrane of most Gram-negatives although labile, in physicochemical terms, is remarkably durable and maintains a degree of integrity even in rather uncomfortable conditions. The pathogenic spirochaetes do not seem to take kindly to discomfort--they do not even relax nicely for electron microscopy in a glassy bed of negative contrast media such as potassium phosphotungstate. The outer envelope components fly apart releasing wall components and often the axial filaments into the hostile world. At best one can say that this dislocation displays some of the macromolecular components of the cell wall. Presumably a lot of periplasmic stuff is released at such times and the physiology and/or physical chemistry of the all-important plasma membrane is severely deranged. The traditionally "delicate" pathogenic spirochaetes may owe this characteristic as much to the instability of these surfaces as to the requirements of intracellular systems which are disrupted as a consequence of handling and change of environment. In this respect many bacteria are not much less difficult to preserve.

Highly ordered macromolecular arrays are, we now find, frequently added on to the formal overcoats of eubacteria and, in fact, practically the whole procaryotic range. These assemblies of proteins (as we learn from studies of the surfaces of Spirillum, Halobacterium, Bacillus, Acinetobacter, etc.), although sometimes cross-linked and covalently bonded to the substrate wall-layer, are often very hard to hold together during preparative procedures because the association is dependent upon either/or both electrostatic and salt bridges. Consequently such layers can be missing from cells washed in media of inappropriate pH or cationic content during fractionation or in embedding for sectioning unless vigilant controls are exercised to recognize and prevent such losses. The dispersion of surface components evident in most preparations of spirochaetes should be a warning to us all and require study of the ionic and other physical requirements for stability.

Because these superficial layers and components are genetically dispensable we are continually asked, "What is the function of these structured layers?" They are an important protection to some, e.g. allowing Spirillum to resist Bdellovibrio predation--and they certainly modify the physi-

cal properties of the surface. This is one type of answer that recognizes selective advantage. In a sense, it is allied to the tried and true function of polysaccharide and polypeptide capsules of bacteria involving restriction of phagocytic defenses of the host. However, we can now appreciate another dimension. Invasive organisms must be able to get close to tissue cells and particularly the barrier at the portal of entry, whether the invasive habit is intercellular or intracellular; in this function the surface of both host cell and parasite is critical and allow the formation of attachments resembling the "tight junctions" between tissue cells.

This juxtaposition of host cell and parasite means, presumably, that the surface components involved require a suitable cationic environment to reduce the charge density on the polyanions so that surfaces can come close together and/or there must be chemically-suitable sites on select macromolecules allowing a more specific union. Both species and regional specificity of attachment to mucosa have been observed for bacteria and one must assume, with more tenuous evidence, that the same applies to spirochaetes. Non-invasive and commensal organisms in array sites must also be well served by such mechanisms which can be considered to delay or avert "washout".

Remarkably little solid information about cell-cell interaction has arisen until recently despite the apparent importance of the mechanisms of specific adsorption and attachment in both the aggregation of cells to form tissues and in host/parasite associations, and the lack of such control in some malignant tumours. As far as bacteria are concerned there is clear evidence that fimbriae (pili) are an attribute of pathogenic strains of gonococcus, among many organisms,and are directly involved in adherence to mucous membranes and are an attribute of many that have to live in that kind of environment. There is also a degree of specificity for the tissue being attacked. Fimbriae are assemblies of polypeptides forming tubular hairs erected on the surface. There is some evidence that material of a similar specificity may be deployed on the cell surface and indicate that alternative arrangements are possible while retaining the physical requirements for adherence. Perhaps fimbriae become of overriding importance when the structure has to poke through a capsule. However, bacterial attachment is not necessarily mediated by fimbriae; cell-cell associations of all types generally require recognition molecules and receptors (e.g. plant lectins and phage receptors).

It would seem that the pathogenic spirochaetes must be

adsorbed in order to rapidly transgress natural barriers, especially mucous membranes, and that the character of components on or in the envelope is likely to be critical as has proved to be the case for other organisms. Furthermore, the macromolecular components of surfaces represent, in different terms, the description of the antigenic mosaic that the organism presents to the world. The mutability of these surface components is legendary, as the characteristics of the Borrelia of the relapsing fevers attest.

The most unique structural feature of some Treponema is the presence of a bundle of fine tubules, which parallel the flagellar filaments and lie inside the plasma membrane. You could not be more interested than I in hearing further of the nature and distribution of these. They are, as far as I know, the only such example in the procaryotes and are tantalizing in their anatomic associations. Of course, in eucaryotes the microtubules (though they may be larger, more rigid) are related in some mysterious way to cytoplasmic streaming and the ballet of mitosis neither of which are operative of procaryotic cells. We shall have fun speculating. I can assure you that several of us have looked for microtubules without success in other bacteria - this may just mean that we are not providing appropriate circumstances for their preservation and demonstration.

Comparative cytology is still developing in strength and scope. The first stage is descriptive involving the accumulation of structural and biochemical data; the second stage is associative bringing the elements together and describing structure in functional terms and the third stage involves synthesis with the integration of molecular, genetic and physiological understanding. There is still a long way to go in the study of spirochaetes (in fact, almost anything except Escherichia coli and Bacillus subtilis) but the papers that follow will lay the basis, and we must appreciate the careful and painstaking effort that goes into such work.

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